REMARKS

The Invention

The invention features E4orf4-encoding nucleic acids, pharmaceutical compositions and expression vectors containing the same, and methods for their use.

The Office Action

Claims 1-60 and 64-80 are pending. Claims 1-12, 14-16, 19-22, 24-26, 29-44, 55-60, and 64-80 are withdrawn as being drawn to a nonelected invention. Claims 13, 17, 18, 23, 27, and 28 are rejected for indefiniteness. Claims 13, 17, 18, 23, 27, 28, 45-48, and 50-54 are rejected for lack of enablement and for being supported by an inadequate written description. Claims 47 and 49-52 are rejected for anticipation by Chroboczek et al. (Virology 186: 280-285, 1992; hereafter "Chroboczek"). Claims 45, 53, and 54 are rejected for obviousness over Ohgi et al. (J. Biochem. 109:776-785, 1991; hereafter "Ohgi") in view of Chroboczek. Claim 48 is rejected for obviousness over Miller et al. (FASEB J 9:190-199, 1995; hereafter "Miller") in view of Chroboczek and Marcellus et al. (J. Virol. 70:6207-6215, 1996; hereafter "Marcellus"). The declaration was deemed defective because there was no signature of Josee Lavoie.

Rejections Under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 13, 17, 18, 23, 27, 28, and 45-54 for lack of enablement. According to the Examiner, the specification is not enabling for (i) *in vivo* gene therapy methods; or (ii) the use of any E4orf4 polypeptide other than that having the sequence of SEQ ID NO.: 3. Applicants note that claims 13, 17, 18, 23, 27, 28, and 45-54 have been canceled and new claims 81-100 have been added. In order to hasten prosecution of these newly-added claims, Applicants provide herewith arguments why these claims should not be rejected for lack of enablement.

Gene therapy

The Examiner states that the claims were not enabled because "the state of the prior art was not well developed and was highly unpredictable at the time of the invention." Following this line of reasoning, the Examiner then asserts that there were numerous obstacles (e.g., lack of efficient delivery systems, lack of sustained expression) to performing clinical gene therapy on humans and, therefore, the claims lack enablement.

New claims 81-100, like the claims rejected for lack of enablement, are directed to methods and reagents for "increasing apoptosis." The Examiner is reading into the claim term "increasing apoptosis" restrictions which the claim does not contain. Increasing apoptosis is simply the augmentation of the number of cells to undergo apoptosis relative to an untreated control. They are not more narrowly drawn to a method of treating or

preventing disease.

Thus, in the present case, the proper determination for enablement is whether one skilled in the art could increase cell apoptosis without undue experimentation.

According to In re Wands, 858 F.2d 731, 737, (Fed Cir. 1988):

> Factors to be considered in determining whether a disclosure would require undue experimentation" include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

The specification teaches a method for increasing apoptosis in a cell by introducing into the cell a nucleic acid encoding an adenoviral E4orf4 protein. Specific methods for introducing the E4orf4-encoding nucleic acid are described on page 36, line 22, to page 37, line 15, and on page 49, line 21, to page 52, line 4, of the specification. Moreover, as stated in the specification, the expression vectors described on page 50, line 18, to page 51, line 9 (*e.g.*, retroviral vectors, adenoviral vectors, adeno-associated viral vectors) and methods of their use for expressing foreign genes in cell *in vivo* were generally known at the time of filing. It is axiomatic that the specification need not describe, and preferably omits, that which is well-known in the art. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1446 (Fed. Cir. 1986).

One method of expressing an E4orf4 protein in a cell involves the use of adenoviral vectors to express a nucleic acid sequence encoding the E4orf4 protein under the expression of a suitable promoter. Working examples of the use of E4orf4 to increase

apoptosis are provided, for example, on page 29, line 26, to page 31, line 4, of the specification, and Applicants proposed that the same mechanism that increased apoptosis *in vitro* would also increase apoptosis *in vivo*.

To support this contention, Applicants direct the Examiner's attention to the attached Declaration of Philip Branton, Ph.D., an inventor on the above-captioned application. As paragraph 2 of the Declaration notes, an expression vector containing a nucleic acid encoding E4orf4 was administered to mice having subcutaneous implants of H1299 lung carcinoma cells or C33A cervical carcinoma cells. As is shown in Exhibits A and B and summarized in paragraph 3 of the declaration, adenovirally-mediated expression of E4orf4 resulted in reduced tumor growth relative to control mice. The foregoing findings demonstrate that E4orf4 functions *in vivo* to increase apoptosis of tumor cells. Additionally, paragraph 4 of the Declaration demonstrates that the mouse models used in the foregoing example are well-accepted as indicative of success by those skilled in the art for therapeutic use in other mammals, including humans.

E4orf4 Polypeptides

The Examiner asserts that the claims lack enablement because Applicants have not described any E4orf4-encoding nucleic acid having about 50% or greater nucleotide sequence identity with the DNA sequence of SEQ ID NO: 3. Applicants respectfully disagree.

At pages 10 and 11 of the specification, Applicants state that E4orf4 polypeptides

from adenoviral serotypes other than Ad2 serotype are included in the invention. These polypeptides can be encoded by nucleotide sequences that have about 50% identity to that of Ad2. The attached declaration of Dr. Branton demonstrates this: the nucleotide sequences from Ad3, Ad9, Ad12, and Ad40 serotypes encoding E4orf4 share 44%, 53%, 51%, and 48% with Ad2 E4orf4 (paragraph 6). Despite having 50% identity, three of the four E4orf4 polypeptides induce apoptosis at levels comparable to that induced by Ad2 E4orf4 (paragraph 7 and Exhibit C). Moreover, the only E4orf4 polypeptide that does not induce apoptosis, Ad3, is expressed at lower levels than the others, indicating that it may be insufficient expression, and not the polypeptide itself, that is the cause of the failure of the polypeptide to induce apoptosis.

Applicants also predicted that some amino acids could be altered without a loss in biological activity. At page 40, line 23, to page 41, line 25, Applicants describe methods for producing E4orf4 polypeptides having conservative or non-conservative amino acid substitutions. These modified polypeptides would, of course, be encoded by nucleic acid molecules that are <u>not</u> identical to wild-type sequence. As is described in paragraph 8 of the attached declaration of Dr. Branton, the following changes have been shown to <u>not</u> alter E4orf4 biological activity: P10A, C18A, Y26A, D31A/V32A/R34A, H38A, Y42A, E44A, P45A, E46A/R48A, R48A, Y59A, C78A, C85A, D99A, and S106A (the first letter is the wild-type amino acid from Ad2, the number is the residue, and the last letter is the substituted amino acid).

Finally, Applicants have added new claims to nucleic acids capable of hybridizing

at high stringency to the complement of the nucleic acid of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis. Methods for determining whether a nucleic acid would satisfy these criteria are well known and described at page 11, lines 15-21, and page 22, lines 19-27, of the specification.

Thus, by combining the teachings of the specification with standard techniques known at the time of filing to one of ordinary skill in the art, a practitioner could clearly make and test nucleic acid molecules having about 50% or greater identity to the disclosed sequences or being capable of hybridizing at high stringency to the complement of the nucleic acid of SEQ ID NO.: 3 without undue experimentation. Moreover, one would have a reasonable expectation that such experimentation would successfully result in achieving the desired goal: a nucleic acid molecule encoding a polypeptide having the appropriate biological activity.

In effect, then, restricting Applicants' claims to the specific nucleic acid sequences disclosed in the application would allow a competitor to circumvent the claims and use Applicants' discovery without providing compensation. This would be manifestly unfair. Accordingly, this rejection should be withdrawn.

In view of the foregoing remarks, Applicants respectfully request that the rejection of the claims for lack of enablement be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 13, 17, 18, 23, 27, and 28 were rejected for omitting essential steps.

Applicants now cancel these claims, and add new claims 81-101. Applicants submit that these new claims particularly point out and distinctly claim the invention.

Rejections Under 35 U.S.C. § 102(b)

Claims 47 and 49-52 were rejected for anticipation by Chroboczek. Applicants now cancel claims 47 and 49-52, and this rejection may now be withdrawn. The relevance of Chroboczek to new claims 81-101 is described in the following section.

Rejections Under 35 U.S.C. § 103(a)

Claims 45, 53, and 54 were rejected for obviousness over Ohgi in view of Chroboczek. Applicants now cancel claims 47 and 49-52, and add new claims 81-101. Like canceled claims 45, 53, and 54, new independent claims 88 and 89 (as well as dependent claims 90-94) are directed to pharmaceutical compositions. For the reasons provided below, Applicants submit that these claims are not obvious in view of Ohgi and Chroboczek.

The M.P.E.P. § 2143 states that to establish a *prima facie* case of obviousness, the prior art references must teach or suggest all of the claim limitations. As is described below, the two references cited by the Examiner do not do so.

Claims 88 and 89 are as follows.

> 88. A pharmaceutical composition comprising (i) a substantially purified nucleic acid capable of hybridizing at high stringency to the complement of the nucleic acid of SEQ ID NO.: 3 and encoding a

polypeptide capable of inducing apoptosis, and (ii) a pharmaceutically acceptable carrier, wherein said nucleic acid is operably linked to a heterologous regulatory sequence for expression of said polypeptide in a mammalian cell.

89. A pharmaceutical composition comprising (i) a nucleic acid having 50% or greater nucleotide sequence identity to the nucleotide sequence of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis, and (ii) a pharmaceutically acceptable carrier, wherein said nucleic acid is operably linked to a heterologous regulatory sequence for expression of said polypeptide in a mammalian cell.

Thus, each of these claims requires that the polypeptide-encoding nucleic acid be operably linked to a heterologous regulatory sequence for expression of the polypeptide in a mammalian cell. Such a regulatory sequence is neither taught nor suggested by either references cited by the Examiner. Ohgi describes the expression of a fungal RNase in a yeast cell using a yeast glyceraldehyde 3-phosphate dehydrogenase promoter. Chroboczek describes the sequencing of genome of Ad5 serotype and its comparison with the genome of Ad2 serotype. Like Ohgi, Chroboczek fails to teach or suggest a heterologous regulatory sequence for expression of the polypeptide in a mammalian cell. Moreover, neither of the references provides a motivation to even express E4orf4 in a mammalian cell. Until Applicants' discovery that E4orf4 alone was capable of increasing apoptosis, no function related to mammalian cells was ascribed to E4orf4. Without any appreciation of a function for E4orf4 in mammalian cells, it follows that there would have been no motivation to operably link an E4orf4-encoding nucleic acid to heterologous regulatory sequence for expression of the polypeptide in a mammalian cell. For this reason, new claims 88-94 are novel and nonobvious over Ohgi and Chroboczek.

Claim 48 was rejected for obviousness over Miller, in view of Chroboczek and Marcellus. Applicants submit herewith a Declaration of Dr. Philip Branton, stating that any description in Marcellus was the joint contribution of Philip Branton, Richard Marcellus, Jose Teodoro, and Gordon Shore, each of whom is an inventor in the above-captioned case, notwithstanding the inclusion of the additional authors, who contributed to other work described in this paper. By submission of this Declaration, Applicants are not conceding that the pending claims would have been anticipated by or obvious in view of Marcellus. In view of this Declaration, Applicants request that this rejection be withdrawn.

Like claims 88-94, new claims 81-87 and 95-101 also require that an E4orf4-encoding nucleic acid be operably linked to heterologous regulatory sequence for expression of the polypeptide in a mammalian cell. For the reasons provided above, these claims are also novel and nonobvious over any combination of Ohgi, Chroboczek, Miller, and Marcellus.

PTO-892 Form

Applicants request that Chroboczek, cited by the Examiner in the Examiner's Action, be included on a PTO-892 form.

Conclusion

Applicants submit that the claims are now in condition for allowance, and such

action is respectfully requested. Enclosed is a petition to extend the period for replying for three months, to and including July 18, 2001. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: July 17, 2001

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Marked-up version of claims

Claims 81-100 are new and have no markings.

81. (New) A method of inducing apoptosis of a cell, said method comprising expressing in said cell a nucleic acid having 50% or greater nucleotide sequence identity to the nucleotide sequence of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis, said nucleic acid operably linked to a heterologous regulatory sequence for expression of said polypeptide, wherein expressing said nucleic nucleic acid in said cell induces apoptosis of said cell.

82. (New) The method of claim 81, wherein said nucleotide sequence identity is 75% or greater to the nucleotide sequence of SEQ ID NO.: 3.

83. (New) The method of claim 82, wherein said nucleotide sequence identity is 90% or greater to the nucleotide sequence of SEQ ID NO.: 3.

84. (New) A method of inducing apoptosis of a cell, said method comprising expressing in said cell a nucleic acid capable of hybridizing at high stringency to the complement of the nucleic acid of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis, said nucleic acid operably linked to a heterologous regulatory sequence for expression of said polypeptide, wherein expressing said nucleic acid in said cell induces apoptosis of said cell.

85. (New) The method of claim 81 or 84, wherein said regulatory sequence is capable of expressing said nucleic acid in a constitutive, inducible, or cell-type specific manner.

86. (New) The method of claim 81 or 84, wherein said nucleic acid is in an adenoviral vector or a retroviral vector.

87. (New) The method of claim 81 or 84, wherein said cell is a cancer cell.

88. (New) A pharmaceutical composition comprising (i) a substantially purified nucleic acid capable of hybridizing at high stringency to the complement of the nucleic acid of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis, and (ii) a pharmaceutically acceptable carrier, wherein said nucleic acid is operably linked to a heterologous regulatory sequence for expression of said polypeptide in a mammalian cell.

89. (New) A pharmaceutical composition comprising (i) a nucleic acid having 50% or greater nucleotide sequence identity to the nucleotide sequence of SEQ ID NO.: 3

and encoding a polypeptide capable of inducing apoptosis, and (ii) a pharmaceutically acceptable carrier, wherein said nucleic acid is operably linked to a heterologous regulatory sequence for expression of said polypeptide in a mammalian cell.

90. (New) The composition of claim 89, wherein said nucleotide sequence identity is 75% or greater to the nucleotide sequence of SEQ ID NO.: 3.

91. (New) The composition of claim 90, wherein said nucleotide sequence identity is 90% or greater to the nucleotide sequence of SEQ ID NO.: 3.

92. (New) The composition of claim 88 or 89, wherein said regulatory sequence is capable of expressing said nucleic acid in a constitutive, inducible, or cell-type specific manner.

93. (New) The composition of claim 88 or 89, wherein said nucleic acid is in an adenoviral vector or a retroviral vector.

94. (New) The composition of claim 88 or 89, wherein said nucleic acid encodes a polypeptide having a conservative amino acid substitution relative to the amino acid sequence of SEQ ID NO.: 4.

95. (New) An expression vector comprising a nucleic acid capable of hybridizing at high stringency to the complement of the nucleic acid of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis, wherein said nucleic acid is operably linked to a heterologous regulatory sequence for expression of said polypeptide in a mammalian cell.

96. (New) An expression vector comprising a nucleic acid having 50% or greater nucleotide sequence identity to the nucleotide sequence of SEQ ID NO.: 3 and encoding a polypeptide capable of inducing apoptosis, wherein said nucleic acid is operably linked to a heterologous regulatory sequence for expression of said polypeptide in a mammalian cell.

97. (New) The expression vector of claim 96, wherein said nucleotide sequence identity is 75% or greater to the nucleotide sequence of SEQ ID NO.: 3.

98. (New) The expression vector of claim 97, wherein said nucleotide sequence identity is 90% or greater to the nucleotide sequence of SEQ ID NO.: 3.

99. (New) The expression vector of claim 95 or 96, wherein said regulatory sequence is capable of expressing said nucleic acid in a constitutive, inducible, or cell-

type specific manner.

100. (New) The expression vector of claim 95 and 96, wherein said expression vector is an adenoviral vector or a retroviral vector.